HHS Public Access

Author manuscript

Nucl Med Biol. Author manuscript; available in PMC 2015 October 26.

Published in final edited form as:

Nucl Med Biol. 2013 April ; 40(3): 361–365. doi:10.1016/j.nucmedbio.2012.11.011.

Equivalence of Arterial and Venous Blood for [^{11}C]CO_2-Metabolite Analysis Following Intravenous Administration of 1-[^{11}C]Acetate and 1-[^{11}C]Palmitate

Yen Ng[a,d], **Steven P. Moberly**[b], **Kieren J. Mather**[c], **Clive Brown-Proctor**[d], **Gary D. Hutchins**[d], and **Mark A. Green**[d]

[a]Department of Industrial and Physical Pharmacy, Purdue University, West Lafayette, IN 47907

[b]Department of Cellular and Integrative Physiology, Indiana University School of Medicine, Indianapolis, IN 46202

[c]Department of Medicine, Indiana University School of Medicine, Indianapolis, IN 46202

[d]Department of Radiology and Imaging Sciences, Indiana University School of Medicine, Indianapolis, IN 46202

Abstract

Purpose—Sampling of arterial blood for metabolite correction is often required to define a true radiotracer input function in quantitative modeling of PET data. However, arterial puncture for blood sampling is often undesirable. To establish whether venous blood could substitute for arterial blood in metabolite analysis for quantitative PET studies with 1-[^{11}C]acetate and 1-[^{11}C]palmitate, we compared the results of [^{11}C]CO_2-metabolite analyses performed on simultaneously collected arterial and venous blood samples.

Methods—Paired arterial and venous blood samples were drawn from anesthetized pigs at 1, 3, 6, 8, 10, 15, 20, 25 and 30 minutes after i.v. administration of 1-[^{11}C]acetate and 1[^{11}C]palmitate. Blood radioactivity present as [^{11}C]CO_2 was determined employing a validated 10-minutes gas-purge method. Briefly, total blood ^{11}C radioactivity was counted in base-treated [^{11}C]-blood samples, and non-[^{11}C]CO_2 radioactivity was counted after the [^{11}C]-blood was acidified using 6N HCl and bubbled with air for 10 minutes to quantitatively remove [^{11}C]CO_2.

Results—An excellent correlation was found between concurrent arterial and venous [^{11}C]CO_2 levels. For the [^{11}C]acetate study, the regression equation derived to estimate the venous [^{11}C]CO_2 from the arterial values was:$y = 0.994x + 0.004$ (r^2=0.97), and for the[^{11}C]palmitate: $y = 0.964x - 0.001$ (r^2=0.9).Over the 1-30 minute period, the fraction of total blood ^{11}C present as [^{11}C]CO_2 rose from 4% to 64% for acetate, and 0% to 24% for palmitate. The rate of [^{11}C]CO_2

Correspondence: Mark A. Green, Ph.D. Department Radiology and Imaging Sciences, Indiana University School of Medicine, R2, E-124, 950 West Walnut Street, Indianapolis, IN 46202-5181, Phone: 317-278-9844, magreen@iupui.edu.

appearance in venous blood appears similar for the pig model and humans following i.v. [^{11}C]-acetate administration.

Conclusion—Venous blood [^{11}C]CO_2 values appear suitable as substitutes for arterial blood samples in [^{11}C]CO_2 metabolite analysis after administration of [^{11}C]acetate or [^{11}C]palmitate.

Advances in Knowledge and Implications for Patient Care—Quantitative PET studies employing 1-[^{11}C]acetate and 1-[^{11}C]palmitate can employ venous blood samples for metabolite correction of an image-derived tracer arterial input function, thereby avoiding the risks of direct arterial blood sampling.

Keywords

arterial; venous; $^{11}CO_2$; metabolite; acetate; palmitate

1. Introduction

Simple carboxylic acids radiolabeled with carbon-11 ($t_{1/2}$: 20.4 min), especially [^{11}C]acetate and [^{11}C]palmitate, have been used widely as tools for imaging with positron emission tomography (PET) to evaluate the rate at which their endogenous counterparts are being oxidatively metabolized by myocardium under various physiological conditions. One of the major radiolabeled metabolites produced by these compounds is [^{11}C]CO_2, a product of the tricarboxylic acid cycle. Models for quantification of myocardial metabolic rate from the resulting PET data require knowledge of both the concentration of radioactivity in myocardium as a function of time, as well as knowledge of the concentration of radiopharmaceutical in the arterial blood supplying that tissue. While the PET images readily quantify radioactivity in the myocardium, as well as the arterial blood radioactivity (based on a region of interest in the cavity of the left ventricle or left atrium), those PET data simply define regional radionuclide concentrations without information on the chemical form of the radionuclide. Thus, for tracer kinetic modeling to quantify metabolic rates, it is necessary to independently measure the fraction of total blood ^{11}C activity present in blood as [^{11}C]CO_2 to generate a correct radiopharmaceutical arterial input function.

The technique most commonly used to determine the radiolabeled CO_2 concentration of blood is simple*in vitro* acidification of a blood sample, followed by purging with an inert gas to drive off CO_2. The fraction of total blood ^{11}C activity that remains after the gas purge represents ^{11}C species other than $^{11}CO_2$. This method has been used in a number of preclinical and clinical PET studies yielding important insights into myocardial metabolism. Here we report experimental validation of the selectivity of the gas-purge technique for removing $^{11}CO_2$ from blood. Since direct arterial cannulation for blood sampling is invasive, painful, and can result in undesirable complications, we have then applied this analytical method to assess whether arterial blood [^{11}C]CO_2 concentrations following administration of [^{11}C]acetate and [^{11}C]palmitate can be accurately, and less invasively, estimated by the [^{11}C]CO_2 levels of venous blood.

2. Materials and Methods

2.1 General

The [^{11}C]CO_2, 1-[^{11}C]acetate, and 1-[^{11}C]palmitate radiotracers were obtained from the Radiochemistry Core of the Indiana Institute for Biomedical Imaging Sciences (IIBIS). The 1-[^{11}C]acetate and 1-[^{11}C]palmitate tracers were prepared following the published methods. The [^{11}C]CO_2 in aqueous solution was obtained by dissolving the no-carrier-added [^{11}C]CO_2 gas in a 2 mL sodium hydroxide (100mM) solution. The resultant sodium ^{11}C-bicarbonate solution was then mixed with 3 mL of saline. The final solution contained 10-15 mCi/mL (370-555 MBq/mL). Theheparanized blood samples used for the air purge validation study were taken from the arterial line of swineprior to initiation of planned PET studies. Heparinized blood samples for the arterial-venous comparison study were obtained from four mature miniature pigs (male; ∼25kg) at specified times after sequential injection of 1-[^{11}C]acetate and 1-[^{11}C]palmitate(allowing ∼90-minutes between injections for decay of the prior dose). Arterial blood samples were drawn from the carotid artery, while venous blood was sampled from the jugular vein. The pigs were anesthetized with 1-3% isoflurane and mechanically ventilated throughout the period of the study. The protocol for the animal studies was approved by the Indiana University Animal Care and Use Committee. Radioactivity measurements were made by dose calibrator or NaI(Tl) automatic gamma counter (Beckman Gamma 8000).

2.2 Validation of protocol for the determination of [^{11}C]CO_2 in blood

Radioactive sample handling was carried out behind lead-shielding in a chemical fume hood. A 50-100 μCi sample (20-100 μL) of aqueous [^{11}C]CO_2 or [^{11}C]acetate or [^{11}C]palmitate/HSA was added to 3 mL of blood *in vitro* and gently mixed. A 1 mL aliquot of the blood was then transferred to a counting vial containing 1 mL sodium hydroxide(0.1 N) and 3 mL isopropanol, which was then immediately capped to retain any $^{11}CO_2$ as bicarbonate. This base-treated blood contained all ^{11}C radioactivity (vial A). A second 1 mL aliquot of the blood containing radiotracer was added to a counting vial containing 1 mL sodium bicarbonate (0.5 N) and 3 mL isopropanol, and the mixture was vortex mixed. Then, 1 mL 6N hydrochloric acid was added followed by 10-20 secondsof vortex mixing. Next, the acid-treated blood sample was purged with air,bubbled into the blood via a 4-inch flexible filter straw (B Braun Medical, Inc., Bethlehem, PA), for 10 and 15 minutes to drive off the $^{11}CO_2$ (vial B). The activity remaining in “vial B” represents for total non-$^{11}CO_2$ radioactivity. After decay correction of the radioactivity measurements to a common time point, the percentage of total radioactivity released as $^{11}CO_2$ was calculated as:

$$Radioactivity\ Present\ as\ CO_2 = \frac{Vial\ A\ Radioactivity - Vial\ B\ Radioactivity}{Vial\ A\ Radioactivity} \times 100\%$$

2.3 Determination of [^{11}C]CO2 in paired arterial and venous blood samples afterintravenous administration of [^{11}C]acetate and [^{11}C]palmitate

Paired arterial and venous blood samples (each 2-3 mL) were drawn simultaneously from anesthetized swine into heparinized syringes at 1, 3, 6, 8, 10, 15, 20, 25 and 30 min after i.v.

administration of 1-[^{11}C]acetate (32.2±3.5 mCi; 1.19±0.1 GBq; n=4) or 1-[^{11}C]palmitate (27.5±5.4 mCi;1.0±0.2 GBq; n=4). For each time point, a 1 mL aliquot of the blood was transferred to a counting vial containing 1 mL 0.1 N sodium hydroxide and 3 mL isopropanol, and then immediately capped to retain any $^{11}CO_2$ as bicarbonate (vial A). A second 1 mL aliquot of each blood sample was added to a counting vial containing 1 mL of 0.5 N sodium bicarbonate and 3 mL isopropanol. Then, 1 mL of 6N hydrochloric acid was added, and the acid-treated blood was vortex mixed.The mixture was then bubbled with air for 10 minutes to release $^{11}CO_2$ (vial B). The ^{11}C radioactivity in each vial was then measured with an automatic gamma counter, and after decay correction to a common time point, the fraction of total blood ^{11}C present as [^{11}C]CO_2 was calculated as:

$$Fraction\ of\ Total\ Blood\ Radioactivity\ Present\ as\ ^{11}CO_2 = \frac{Vial\ A\ Radioactivity - Vial\ B\ Radioactivity}{Vial\ A\ Radioactivity}$$

2.4 Analysis of blood ^{11}CO2 after [^{11}C] acetate administration to humans

In conjunction with an IRB-approved study of [^{11}C]acetate PET imaging in humans, the levels of [^{11}C]CO_2 were also determined in peripheral venous human blood samples. The blood was drawn from a forearm vein into heparinized syringes at 1, 3, 6, 8, 10, 15, 20, 25 and 30 min (2-3 mL each) following i.v. administration of 1-[^{11}C]acetate (876.9±140.6 MBq;3 men and 3 women) and immediately analyzed following the procedures described above.

3. Results and Discussion

To verify the suitability of the gas purge method for the determining the level of blood-borne [^{11}C]CO_2, analysis was carried out after directly mixing [^{11}C]CO_2 with whole blood *in vitro*. After acidification and air bubbling through the sample for 10-minutes, more than 99% of the ^{11}C was released from the [^{11}C]CO_2-spiked whole blood (Table 1). Continuation of the gas purge for an additional 5-minutes did not appreciably alter the residual blood ^{11}C level. As expected, blood samples similarly spiked with [^{11}C]acetate or [^{11}C]palmitate showed no loss of radioactivity, even after 15 min of air purging (Table 1). These results are similar to previous reported findings, where 95% of [^{11}C]acetate was retained, and 98% of [^{11}C]-labeled bicarbonate was eliminated, in acid-treated blood samples after 10-minutes of inert gas bubbling. This validation study confirmed that the gas purge method was quite effective for selectively releasing [^{11}C]CO_2 from acid-treated whole blood. Additionally, the analysis method is technically straightforward, simple to implement, and can readily be completed on a time frame compatible with the 20-minute ^{11}C half-life.

To evaluate whether venous blood sampling might reliably substitute for arterial blood sampling as this method is translated to support quantitative PET studies of acetate and palmitate metabolism in humans, we wanted to examine the correlation between arterial and venous blood [^{11}C]CO_2 concentrations after i.v. administration of [^{11}C]acetate or [^{11}C]palmitate. The kinetic models for quantification of tissue metabolism with [^{11}C]acetate and [^{11}C]palmitate require knowledge of the true tracer arterial blood time-activity curve, but direct arterial cannulation for blood sampling is undesirably invasive in human studies.

Reliance solely on the PET data for quantification of the total arterial blood level of radioactivity, coupled with venous blood sampling for metabolite analysis, is far more practical to implement in clinical studies. But, this latter approach is only valid if venous blood can be demonstrated to be a reasonable surrogate for quantifying the relative levels of parent tracer and metabolite(s) in arterial blood.

Paired arterial and venous blood samples were concurrently collected at 1, 3, 6, 8, 10, 15, 20, 25 and 30 minutes after i.v. administration of either 1-[^{11}C]acetate and 1-[^{11}C]palmitate in four mini pigs at rest. (The length of each imaging session was 30-minutes, setting the final blood sampling time.) Each of the collected blood samples was analyzed immediately to determine the fraction of total blood radioactivity present as [^{11}C]CO_2.

An excellent correlation was found between the arterial and venous blood [^{11}C]CO_2 concentrations at all times following [^{11}C]acetate injection, with a correlation coefficient, r^2=0.97 (n=34 pairs; Fig. 1A). For ^{11}C-palmitate, there is also a good correlation between the measured fractions of radioactivity present as [^{11}C]CO_2 in arterial and venous blood (r^2= 0.9;n=26 pairs; Fig. 1B). Over the 1-30 minute period, the fraction of total blood ^{11}C present as $^{11}CO_2$rose from 4% to 64% for acetate, and from 0% to 24% for palmitate (Fig. 2). However, comparing [^{11}C]acetate to [^{11}C]palmitate, there is a marked difference in the rates at which total blood radioactivity comes to be dominated by the [^{11}C]CO_2 metabolite.

The concordance of the metabolite data for these paired venous and arterial blood samples has a significant impact on imaging study design. For PET studies to quantify the rates of tissue metabolism of [^{11}C]acetate and/or [^{11}C]palmitate, venous blood sampling will be adequate for determining the [^{11}C]CO_2 metabolite correction required in an image-derived arterial blood ^{11}C time-activity curve. Since $^{11}CO_2$ is exhaled, the simple mass balance obviously requires that arterial blood have a lower $^{11}CO_2$ concentration than the venous blood entering the lungs (Fig.3). Our results, however, indicate the fractional loss of $^{11}CO_2$ from the labeled blood pool of CO_2/H_2CO_3 is sufficiently small that blood samples from the vein remain a reasonable surrogate for defining the arterial blood level of the [^{11}C]CO_2 metabolite after administration of 1-[^{11}C]acetate and 1-[^{11}C]palmitate.

Arterial blood time-radioactivity curves showed similarly rapid clearance of both 1-[^{11}C]acetate and 1-[^{11}C]palmitate (Fig. 4). In the myocardium, [^{11}C]acetate is converted to acetyl-CoA, and immediately enters the tricarboxylic acid cycle, where radioactive CO_2 is produced as its end product. The fraction of blood ^{11}C present as [^{11}C]CO_2 in arterial/ venous increased over time steadily, with the absolute [^{11}C]CO_2 concentration reaching a peak at about 10-15 min post injection, and then remaining stable until the end of imaging at 30 minutes. As shown in Figure 2A, by 15 minutes after [^{11}C]acetate administration, approximately 60-70% of the blood radioactivity was labeled bicarbonate, with the radioactivity of $^{11}CO_2/HCO_3^-$ contributing significantly to the blood radioactivity at even a few minutes post-radiopharmaceutical administration. These results are consistent with previous studies.

In the case of [^{11}C]palmitate, approximately 25-30% of the blood radioactivity was present as $^{11}CO_2$ at 20 min post-administration (Fig. 2A). This result is also similar to previously

reported findings. Unlike 1-[^{11}C]acetate, the 16-carbon 1-[^{11}C]palmitate has a more complicated biochemical fate. Some of the 1-[^{11}C]palmitate undergoes β-oxidation, where a 2-C fragment is released as acetyl-CoA and enters the citric acid cycle for complete oxidation. On the other hand, some of the 1-[^{11}C]palmitate administrated may not undergo immediate oxidation, but instead be stored as triglyceride.

For comparison with the findings in the pig model, the levels of $^{11}CO_2$ in venous blood were also measured in six human subjects after intravenous administration of 1-[^{11}C]acetate(Fig. 2B). The rate at which human blood radioactivity becomes predominantly $^{11}CO_2$ is very similar to the findings in the pig model.

4 Conclusions

The 10-minute gas purge method is quite reliable for removing $^{11}CO_2$ from blood. In modeling to quantify the rate of tissue oxidative metabolism using [^{11}C]acetate and [^{11}C]palmitate, [^{11}C]CO_2 levels measured in venous blood appear suitable as a surrogate for quantifying the metabolite contribution to total ^{11}C-radioactivity in arterial blood.

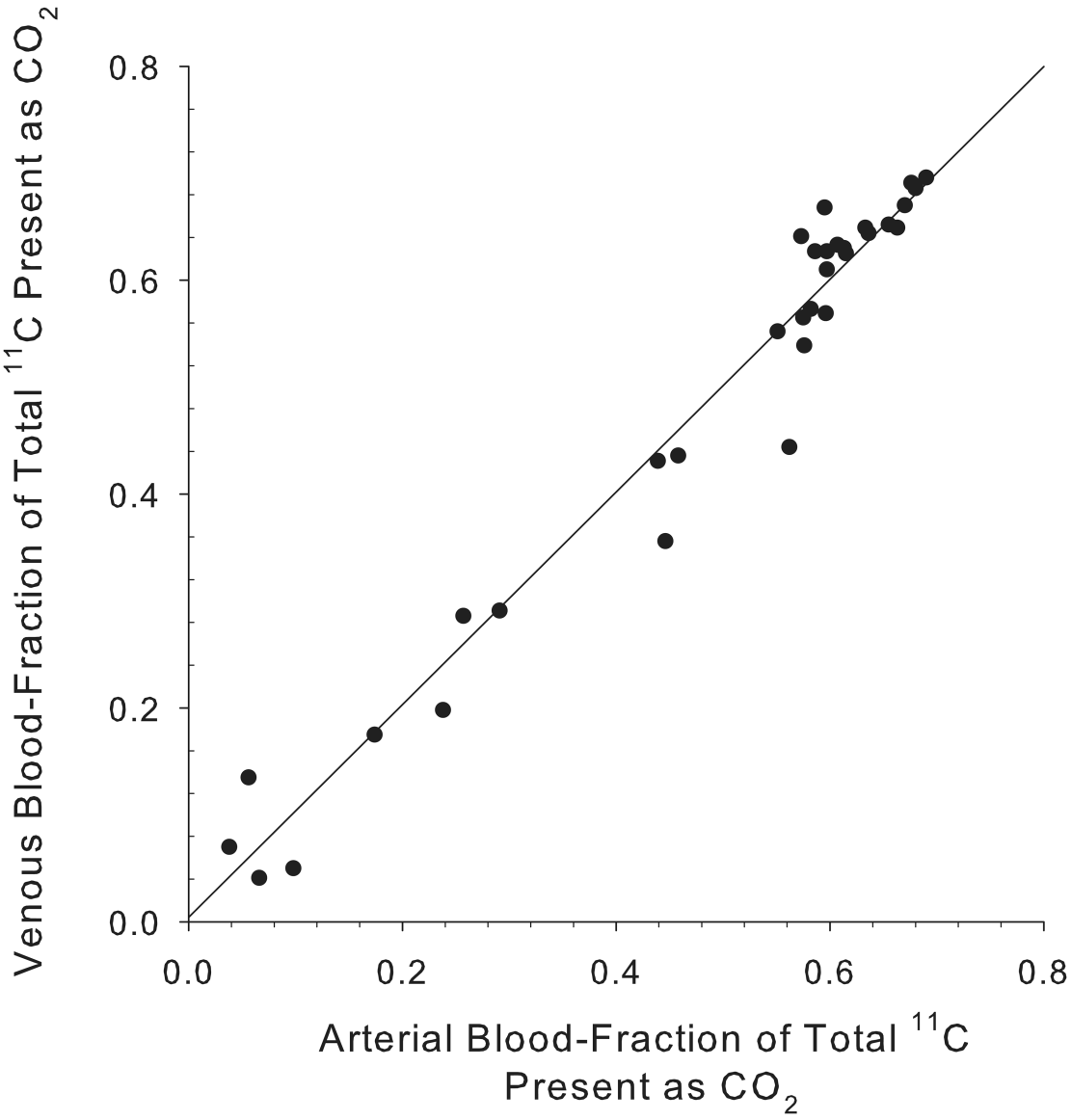
Acknowledgments

Support for this work came from Eli Lilly, NIH (grants NHLBI 092245 and HL 092799), as well as the Indiana Institute for Biomedical Imaging Sciences (IIBIS) core resource of the Department of Radiology, Indiana University School of Medicine. These tracer development and validation efforts were also partially underwritten by the Department of Radiology and Imaging Sciences through the Indiana Institute for Biomedical Imaging Sciences (IIBIS). YN gratefully acknowledges the fellowship support provided by Public Service Department of Malaysia.

References

1. Fox KA, Abendschein DR, Ambos HD, Sobel BE, Bergmann SR. Efflux of metabolized and nonmetabolized fatty acid from canine myocardium. Implications for quantifying myocardial metabolism tomographically. Circ Res. 1985; 57(2):232–43. [PubMed: 4017196]
2. Kisrieva-Ware Z, Coggan AR, Sharp TL, Dence CS, Gropler RJ, Herrero P. Assessment of myocardial triglyceride oxidation with PET and C-11-palmitate. J Nucl Cardiol. 2009; 16(3):411–21. [PubMed: 19212800]
3. Brown MA, Myears DW, Bergmann SR. Validity of estimates of myocardial oxidative-metabolism with C-11 acetate and positron emission tomography despite altered patterns of substrate utilization. J Nucl Med. 1989; 30(2):187–93. [PubMed: 2786932]
4. Walsh MN, Geltman EM, Brown MA, Henes CG, Weinheimer CJ, Sobel BE, et al. Noninvasive estimation of regional myocardial oxygen-consumption by positron emission tomography with C-11 acetate in patients with myocardial-infarction. J Nucl Med. 1989; 30(11):1798–808. [PubMed: 2809744]
5. Clark VL, Kruse JA. Arterial catherization. Crit Care Clin. 1992; 8:687–97. [PubMed: 1327430]
6. Mock BH, Brown-Proctor C, Green MA, Steele B, Glick-Wilson BE, Zheng QH. An automated SPE-based high-yield synthesis of [C-11]acetate and [C-11]palmitate: no liquid-liquid extraction, solvent evaporation or distillation required. Nucl Med Biol. 2011; 38(8):1135–42. [PubMed: 21831651]
7. Brown MA, Myears DW, Bergmann SR. Noninvasive assessment of canine myocardial oxidative-metabolism with C-11 acetate and positron emission tomography. J Am Coll Cardiol. 1988; 12(4): 1054–63. [PubMed: 3262128]
8. Shields AF, Graham MM, Kozawa SM, Kozell LB, Link JM, Swenson ER, et al. Contribution of labeled carbon-dioxide to PET imaging of carbon-11-labeled compounds. J Nucl Med. 1992; 33(4): 581–4. [PubMed: 1552344]

9. Collier TL, Hwang Y, Ramasamy R, Sciacca RR, Hickey KT, Simpson NR, et al. Synthesis and initial evaluation of 17-C-11-heptadecanoic acid for measurement of myocardial fatty acid metabolism. J Nucl Med. 2002; 43(12):1707–14. [PubMed: 12468523]



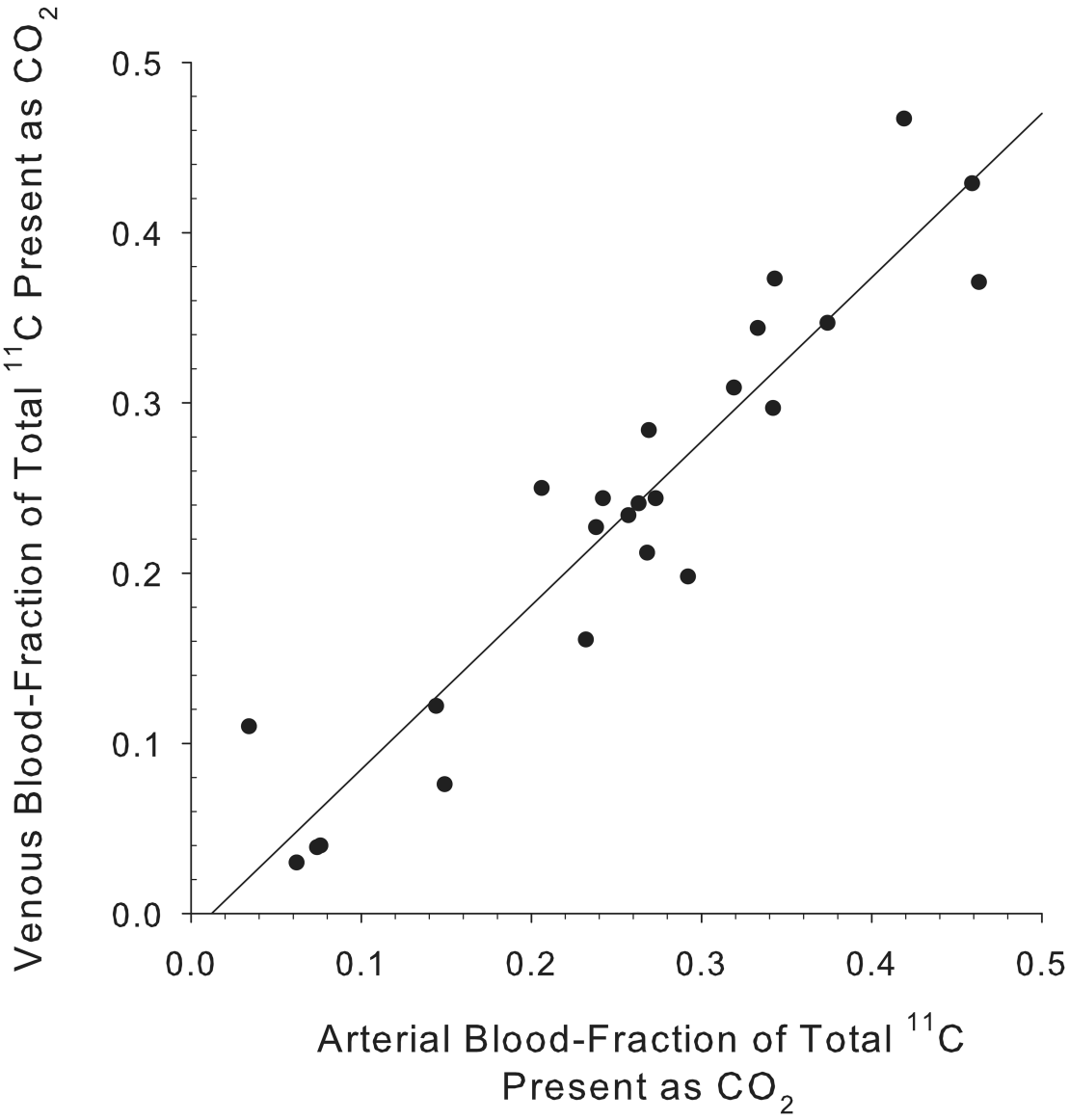
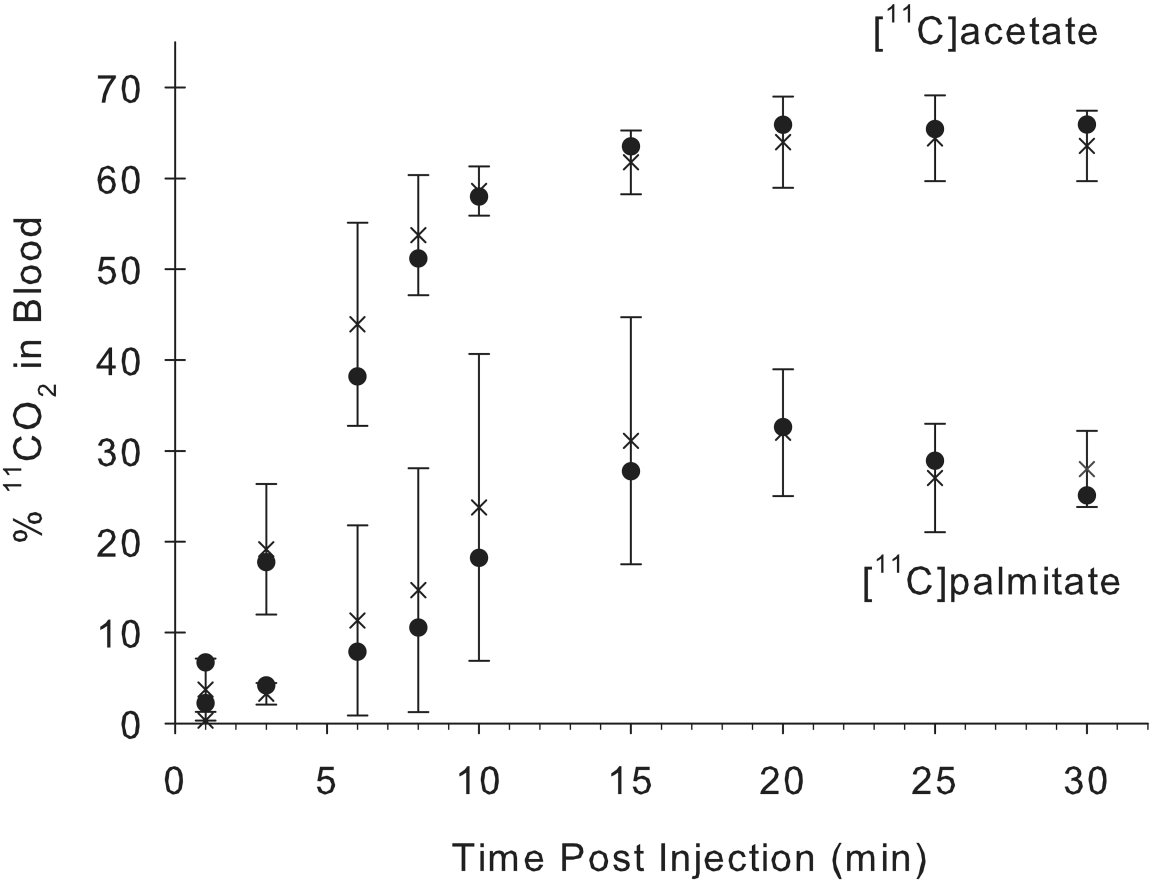


Fig. 1.

Concordance of measured arterial and venous [^{11}C]CO_2 levels. For [^{11}C]acetate (A), the data shown represent a total of 34 paired swine arterial and venous blood samples collected at times: 1, 3, 6, 8,10, 15, 20, 25 and 30 minutes post- i.v injection ($y = 0.994x + 0.004$; $r^2 = 0.97$). At all time points from 1-30 minutes post-injection, venous and arterialblood concentrations of [^{11}C]CO_2 appear essentially identical.For [^{11}C]palmitate (B), the data shown represent 26 paired blood samples collected at 6, 8, 10, 15, 20, 25 and 30 minutes post-i.v injection ($y = 0.964x - 0.011$; $r^2 = 0.9$). Data from one and three minutes post injection of [^{11}C]palmitate are omitted due to the absence of $^{11}CO_2$ in blood.



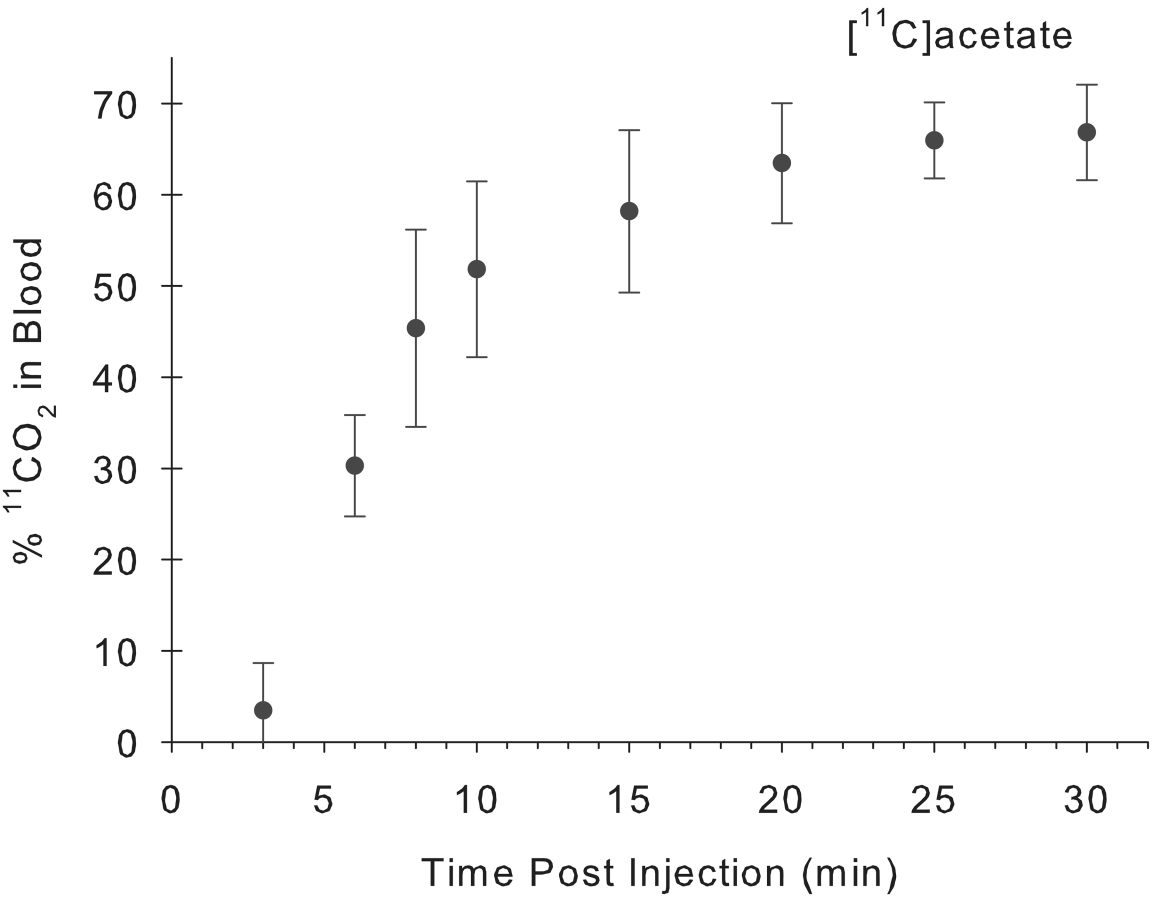
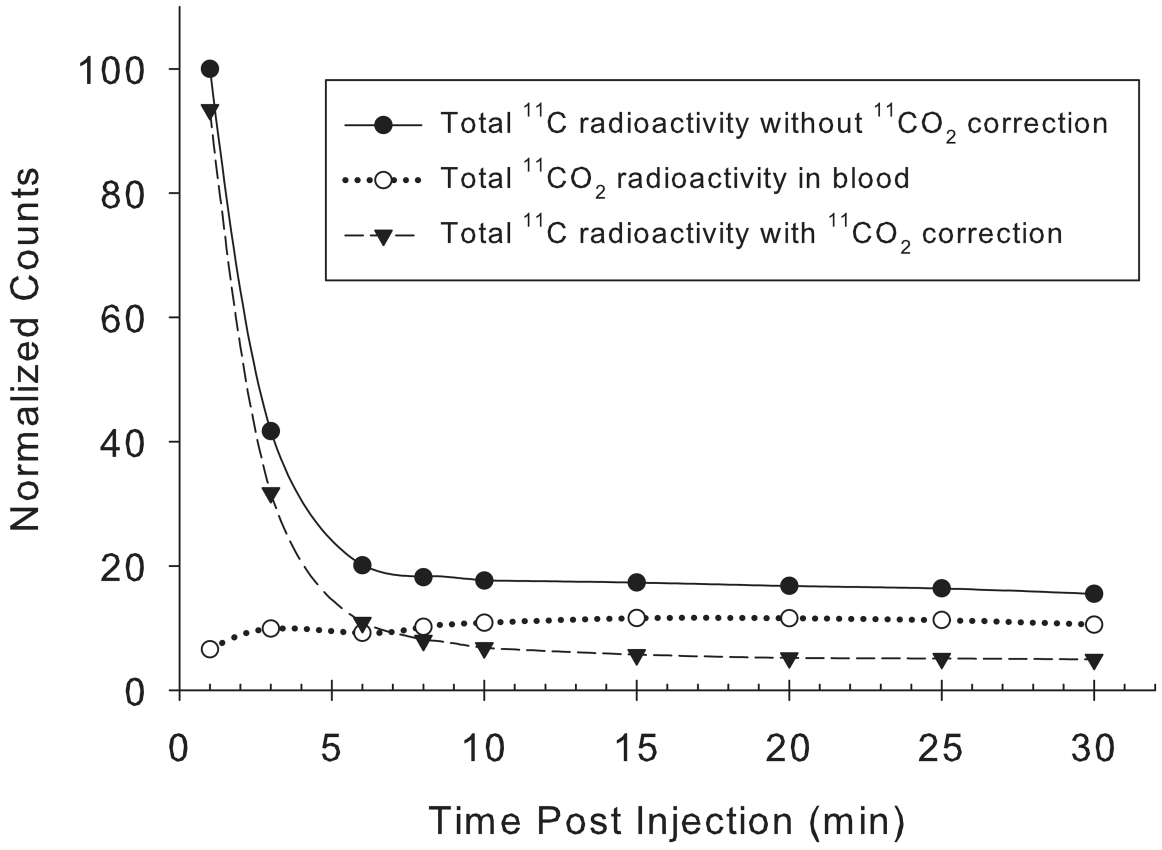


Fig. 2.

Fractions of arterial and venous total blood radioactivity present as $^{11}CO_2$ following i.v. administration of [^{11}C]acetate and [^{11}C]palmitate to miniaturepig (A; n =4), and human (B; n = 6).In pig, the percentage of $^{11}CO_2$ rose from 4% to 64% for acetate, and 0% to 24% for palmitate, over a 30-minute period following the i.v administration of the indicated tracer. The rate of $^{11}CO_2$ appearance in human blood following[^{11}C]acetate administration is very similar to the finding in the pig model. Values given are% $^{11}CO_2$ in blood (mean±S.D.)[x : arterial blood; ● : venous blood].



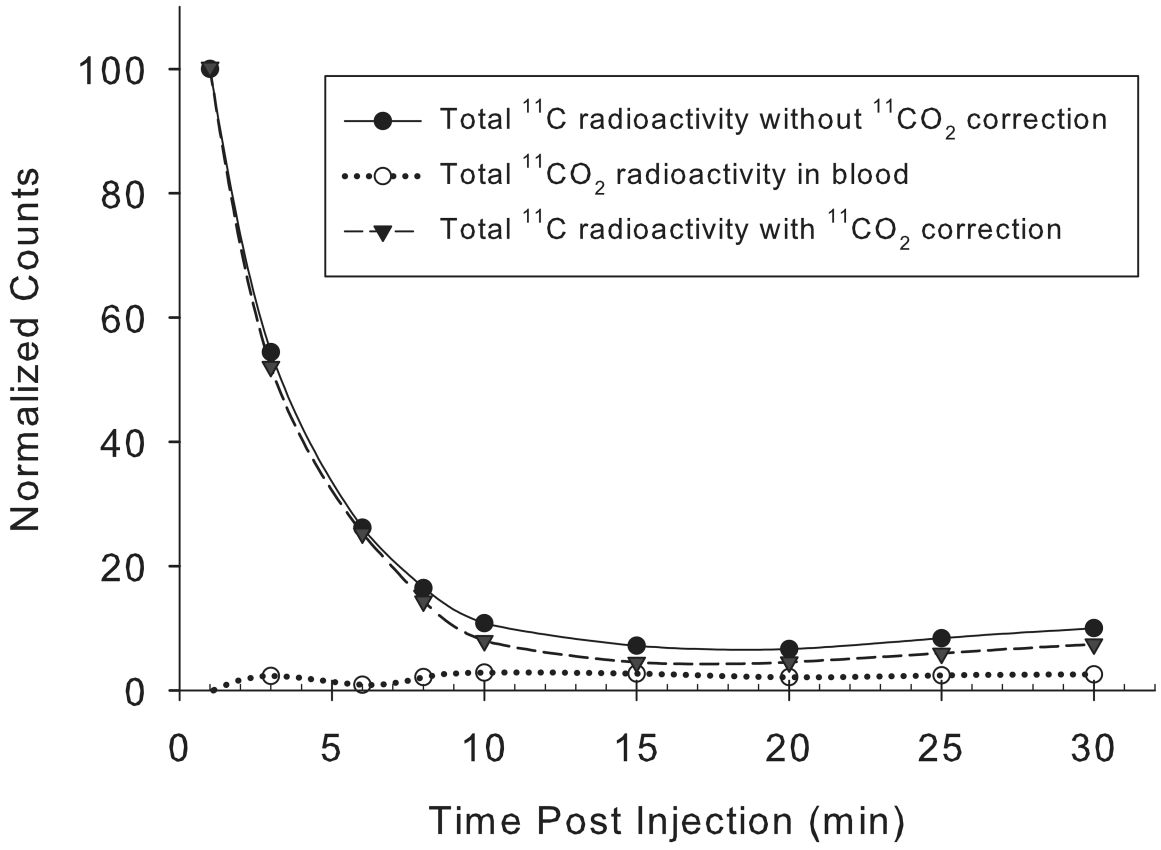


Fig. 3.

While exhaled $^{11}CO_2$ precludes arterial and venous blood from having absolutely identical concentrations of the [^{11}C]CO_2 metabolite of [^{11}C]acetate and [^{11}C]palmitate, the data from this study indicates that venous blood samples remain an excellent surrogate for quantifying the fraction of blood ^{11}C radioactivity present as [^{11}C]CO_2 after i.v. administration of these radiopharmaceuticals.

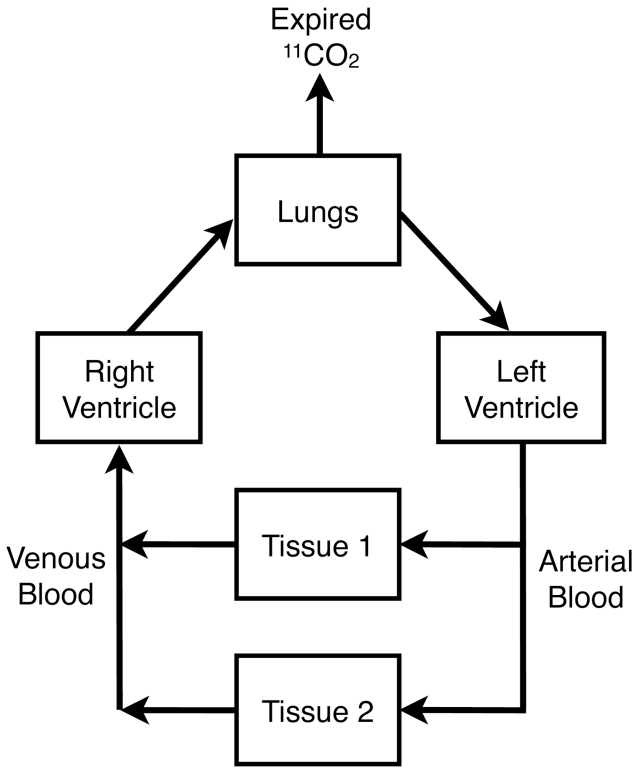


Fig. 4.

Representative normalized decay-corrected arterial blood time-radioactivity curves obtained from a single pig by direct measurement of ^{11}C radioactivity in sampled arterial blood. The [^{11}C]-acetate (A) and [^{11}C]-palmitate (B) are both fairly cleared rapidly from blood, with the [^{11}C]CO_2 metabolite representing a significant fraction of the total radioactivity beyond a few minutes post-injection.

Table 1

Effectiveness of the gas-purge method for selectively removing [^{11}C]CO_2 from acidified whole blood.

Radiopharmaceutical Added to Blood	Percentage of initial ^{11}C lost from blood sample after 10-minute air purge
$^{11}CO_2$	99.8 ± 0.3% (n = 4)
1-[^{11}C]acetate	-0.1 ± 0.5% (n = 3) *
1-[^{11}C]palmitate	-2.4 ± 1.9% (n = 3) *

All results were reported as the mean ± SD for the number of experiments performed (n)

* 15 min of air purge